REVIEW ARTICLE

Waking Up the Sleepers: HIV Latency and Reactivation

*Hoi Ping Mok, Andrew Lever**

In a patient infected with HIV-1, the presence of latently infected cells from which the virus can be reactivated and rekindle HIV infection in the patient necessitates lifelong administration of antiretroviral treatment. The biology of HIV latency and viral silencing is now becoming clearer at a molecular and cellular level. However, our understanding of HIV-1 latency *in vivo* is still inadequate. Attempts to therapeutically reactivate the virus in infected patients have yielded disappointing results. This article reviews the research and clinical findings and discusses current thinking on the subject of HIV latency and reactivation. [*J Formos Med Assoc* 2008;107(12):909–914]

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Since its introduction over 10 years ago and despite the ongoing importance of treatment and prophylaxis of opportunistic infections, highly active antiretroviral therapy (HAART) has become the mainstay of management of patients infected with HIV. HAART uses a combination of antiviral drugs to interrupt the life cycle of the virus and can usually suppress viremia to levels below that detectable by conventional assays, i.e. 50 copies of viral RNA per milliliter (equivalent to 25 viruses since they have dimeric [diploid] genomes). The introduction of HAART in an infected patient is associated with a dramatic drop in viral load within 2 weeks. This is associated with a slower developing, but nonetheless progressive, degree of immune reconstitution. Despite its success, viral eradication from an infected person has never been attained, and thus HAART must be lifelong. Cessation of therapy results in viral reappearance in the circulation within 2 weeks.[1,2]

Viral Reappearance

Where does the recrudescent virus come from? There are two possible sources. One is that very low level viral replication, below the threshold of detection of current assays, persists and can then escape after the cessation of HAART.[3] Two observations support this theory: ultrasensitive techniques can in fact detect very low level viremia in patients treated by HAART and this low level can be further reduced by intensification of antiviral therapy.[4] Secondly, there is evidence that unintegrated viral DNA, a marker of ongoing viral replication, is present in patients treated with HAART.[5] Indeed, on sequential sampling, the detectable DNA sequences of unintegrated (episomal) viruses from patients undergoing suppressive HAART evolve to become drug resistant.[6] Together, these observations suggest that HAART, while inhibiting most viruses or rendering them non infectious, either does not penetrate in sufficient concentrations to

Department of Medicine, University of Cambridge, Cambridge, UK.

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***Correspondence to:** Dr Andrew Lever, Level 5, Addenbrooke's Hospital, Hill's Road, Cambridge, CB2 0QQ, UK.
E-mail: amll1@mole.bio.cam.ac.uk

all sites of virus replication or that it is not 100% inhibitory and that some viruses can still replicate, albeit inefficiently, despite the presence of "therapeutic" blood levels of the drugs.

An alternative source for viral rebound is that it comes from the reactivation of latent viruses. After entering the infected cell, the virus reverse transcribes its RNA genome into double-stranded DNA. The double-stranded viral DNA is incorporated into the host genome, a process termed integration. Subsequently, during virus production, the virus utilizes host machinery for transcription of the integrated proviral genome to produce viral RNA and hence proteins. The integrated provirus genome is a stable molecular form of the virus which will persist and be reproduced and transmitted to each daughter cell at mitosis. This viral DNA may not be transcriptionally active, resulting in a pool of latently infected cells. There is good evidence for this: in cells from infected patients, viral DNA, presumably representing integrated viruses, is detected approximately 10 times more frequently than viral RNA, suggesting that the majority of the integrated viral genomes are not transcribing to produce viral RNA.[7–9] Secondly, viruses recovered in viral rebound after cessation of HAART[10,11] and from patients with low level viremia in the presence of HAART[12] contain drug-sensitive genotypes or archival genotypes that show resistance to previously employed agents and not those used in that patient's most recent HAART. This suggests that these viruses had not been replicating recently but had been reactivated from latency. Thirdly, mathematical modeling of virus turnover during low level viremia under HAART shows minimal viral replenishment,[13] arguing that the stability of the numbers of latent viruses could not be derived from persistence and reseeding (although the detectable viremia could).[14]

Mechanisms of Latency

The cellular biology of HIV latency and silencing is starting to be elucidated. *In vitro* analysis has shown that the major hurdles in the viral life cycle are viral entry into the cell and, subsequently, initiation of viral gene expression.[15] *In vitro* in single round replication assays, the level of unintegrated HIV species peaks at 7 hours post infection but subsequently falls to a low level by 26 hours.[16] Thus, unintegrated HIV species are too unstable to be the dormant molecular form of the virus, and the likely source of latent viruses is silent integrated proviruses.

After integration, viral gene expression bifurcates to either high level gene expression or extinction[17] (once established, viral gene expression is extremely durable—lasting for at least 18 months in one study).[18] Such a reproducibly binary pattern of expression is postulated to be a result of a positive feedback loop mediated by the viral protein Tat, which stimulates further viral gene expression by acting on a responsive element in the viral promoter.[17] Thus, latently infected cells are likely to contain silent, rather than partially expressed, proviruses.

What is the cause of such silencing (see Figure)? *In vitro* studies showed that silencing is observed frequently, even in actively dividing cells,[18,19] suggesting that it may be an intrinsic property of the viral promoter system. The site of provirus integration in the DNA of the cell is potentially important: gene expression from an integrated virus is partially determined by its location in the genome.[20] This site-dependent effect is mediated, at least in part, by the permissiveness of the DNA and DNA binding proteins—the chromatin—at the site of integration. The critical effect of chromatin structure is supported by examination at a local level using techniques such as chromatin histone immunoprecipitation (CHIP) assays[21–23] and can also be inferred from reactivation experiments where pharmacologic agents that alter the chromatin structure can alter the level of viral gene expression.[24,25] The importance of chromatin structure is also apparent from studies of retrovirus-based viral vectors for gene therapy, where the incorporation of a genetic element that can affect the local chromatin can later influence the level of vector gene expression.[26,27] The local

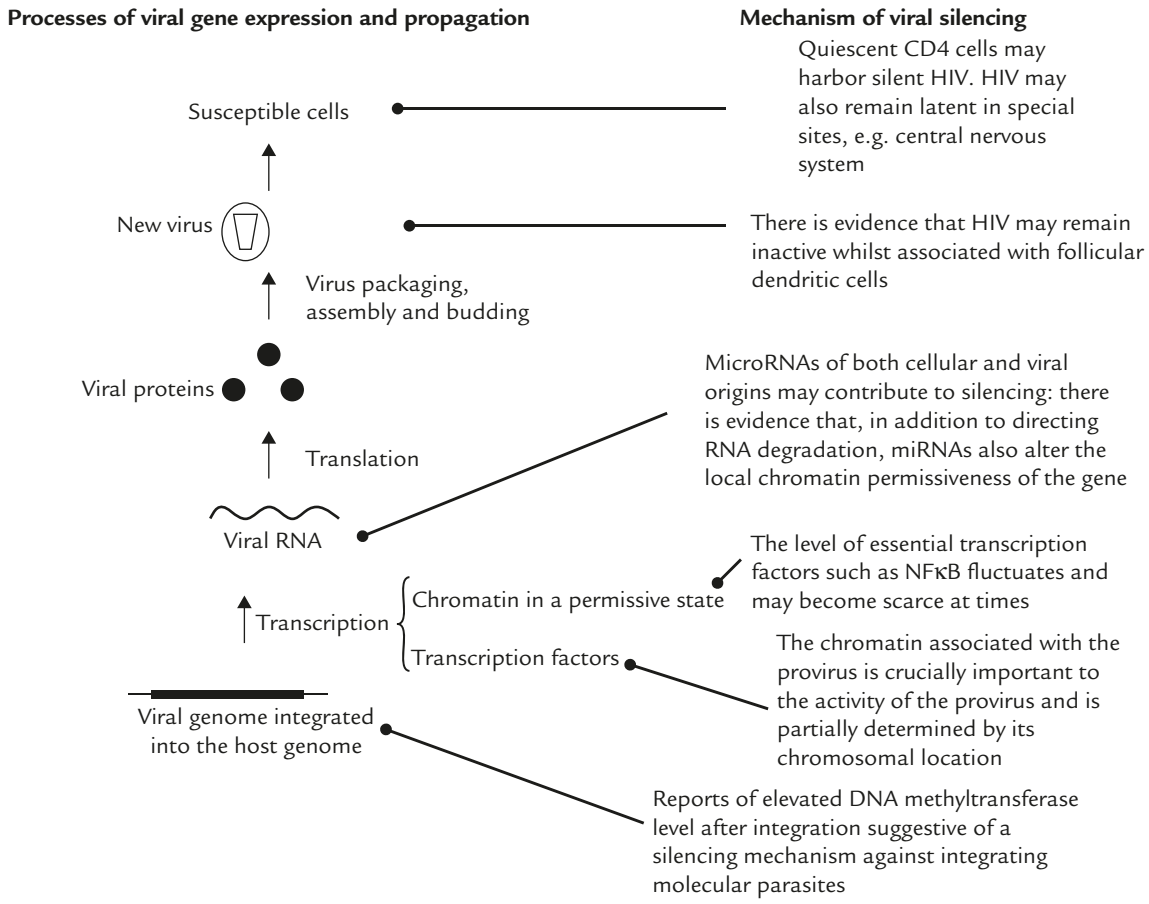

Figure. Processes of viral gene expression and mechanisms of viral silencing.

chromatin structure however is not the sole determining factor. While the level of HIV gene expression increases with higher gene density at the site of integration, very highly expressed regions of the genome exist that are not favored for integration or expression, suggesting the presence of more subtle rules governing the behavior of the provirus.[20] More importantly, variegation—a difference in phenotype within a clonal population—has also been observed, arguing that further modulation can occur within a single cell.[17,18] Therefore, it is also possible that chromatin remodeling is a final common pathway to gene silencing rather than its primary cause. Other factors implicated in proviral silencing include the availability/lack of specific cellular transcription factors. Over 100 transcription factors have been shown to bind to the HIV promoter,[28] of which the effect of NFκB is the best demonstrated. The intracellular level of NFκB fluctuates in a cyclical manner[29] and it is plausible that this could affect the behavior of the provirus. An innate genome defence against invading molecular parasite has also been postulated. There are reports of elevated level of DNA methyltransferase—an enzyme that mediates DNA methylation that leads to tight chromatin repression—after HIV infection.[30] More recently, repression of viral gene expression by viral[31] and cellular[32] microRNA has also been described.

Latency *In Vivo*

The biology of latency *in vivo* is less clear. Studies of latency *in vivo* have been problematic: latently infected cells by definition do not display any protein markers for the cells to be selected for study without their biological characteristics being perturbed.[33] However, the molecular stability achieved by viral integration into the host

genome and the cellular stability of resting CD4 T cells offers an elegant hypothesis: that latently infected cells are generated as the virus infects activated CD4 T cells that are returning to a quiescent state.[34] Indeed, it was possible to detect viral gene products in quiescent CD4 T cells after cellular activation.[34–36] Based on this, many researchers study the latent pool by identifying resting CD4 T cells that produce viral proteins after cytokine stimulation.[33] This approach depends on latent viruses being efficiently reactivated by the chosen stimulatory method and assumes that the clinically important latent pool is in the resting CD4 compartment. The overall picture however suggests something more complex. As discussed earlier, a large proportion of proviruses are silent *in vitro*, even in dividing cells.[19] Early studies performing *in situ* PCR for viral DNA from untreated patients also showed a large proportion of silent proviruses,[7,8] yet latently infected cells identified by cytokine reactivation after tissue harvesting were found infrequently. An association between HIV and follicular dendritic cells in lymphoid tissues in patients treated with HAART has also been demonstrated.[37,38] In addition, the central nervous system has been proposed as a reservoir for latent viruses.[39] Thus, there may be more than one anatomic or tissue compartment contributing to viral latency.

Therapeutic Approaches to Latent Virus

Latently infected cells offer no specific target for antiviral treatment and immunologic clearance and are thus "sanctuary" sites for the virus. A strategy to achieve virus clearance is to therapeutically reactivate latent virus followed by administration of antiviral drugs while also depending on immune clearance of the newly immunologically "visible" infected cells. A number of agents such as histone deacetylate inhibitors trichostatin A,[22,25] prostratin,[40–42] cytokines such as IL-7[43] and TNF-α[44] have demonstrated efficacy *in vitro*. Attempts *in vivo* however have been disappointing. Previous studies combining the cytokine IL-2 with HAART in infected patients showed that viral rebound was the rule after cessation of HAART.[45] More recently, a study using the stimulant of histone deacetylase, valproic acid,[46] *in vivo* caused considerable excitement. The study quantified the latent pool using cytokine stimulation of resting CD4 T cells and found statistically significant reductions in the size of the latent pool in four patients in whom valproic acid was administered. Unfortunately, enfuvirtide was added during the study period to these four patients, making the interpretation of results difficult. Furthermore, a beneficial effect of valproic acid was not seen in patients who were treated with the agent for other indications but did not undergo intensification of therapy with enfuvirtide[47] or in other studies addressing the same issue.[48]

What are the reasons for the disappointing outcomes of experimental therapeutic reactivation? One possibility is that latently infected cells reside in an immunologic sanctuary site or sites inadequately penetrated by HAART. Thus, despite efficient reactivation, the latently infected cells are not eliminated. Another possibility might be that the reactivation regime was ineffective. At present, these remain conjectural, reflecting the paucity of understanding of the nature of the latent reservoir at the tissue level and of what are probably multiple mechanisms involved in the arrest of the virus life cycle at the cellular level.

Future Prospects for Eradicating Latent Virus

How should the hurdle of viral latency be tackled? Clearly, the nature of the latent reservoir *in vivo* needs to be better defined. The assumption that latency is an intrinsic property of the virus, and once reactivated the latently infected cell would be susceptible to immunologic clearance or HAART, should be verified *in vivo*. Further therapeutic reactivation studies could be pursued using a combination of stimuli targeting many or all of the viral silencing mechanisms so far identified to ensure efficient reactivation. The use of

combination stimuli worked well *in vitro* in other retroviruses in overcoming chromatin-mediated latency[49] and also in HIV.[25] However, this may involve a large number of pharmaceutical agents, which may be unacceptably toxic or simply impractical. Perhaps a more modest but achievable approach would be to identify the most important mechanism(s) causing viral latency and to target these selected few for reactivation. This could reduce but would not eliminate the risk of viral rebound. To do this, we require much more detailed knowledge on the relative contribution of each of the silencing mechanisms contributing to virus latency and the nature of the latent reservoir.

Research on drugs targeting viral replication in HIV has been one of the greatest therapeutic successes in any branch of medicine in the last few decades. The next challenge of eliminating the latent virus reservoir is probably an even bigger one but the rewards would be immense—true elimination of HIV from an infected person.

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